

CD44 and E-Cadherin Expression in Primary Epithelial Tumours of Ovary and Comparison with Histological Type and Tumour Differentiation - A Cross-Sectional Study from Thrissur, Kerala

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ABSTRACT

BACKGROUND

Ovarian tumours are the common cause of morbidity and mortality in women worldwide. Primary epithelial ovarian tumours comprise the majority. Cluster differentiation 44 (CD-44) is a trans-membrane glycoprotein which plays a role in cell-cell interaction, adhesion and migration, leading to the progression and metastasis of tumour. E-cadherin is another cell adhesion molecule which plays an important role in neoplastic progression. So, it is necessary to find out the relationship of CD-44 and E-cadherin expression with histological types and tumour differentiation, which might predict the prognosis. The present study was undertaken to assess the pattern of expression of CD-44 and E-cadherin in primary epithelial tumours of ovary and to determine the relationship between their expression with age, histological type and tumour differentiation.

METHODS

This is a cross-sectional study conducted in the Department of Pathology, Government Medical College, Thrissur; a tertiary care institution. Histological types and tumour differentiation for each case was determined from haematoxylin and eosin sections. Immunohistochemical stain for CD-44 and E-cadherin was done. Pattern of expression was studied and a semi quantitative score was calculated. Expression of both markers was then compared with the age, histological type and tumour differentiation.

RESULTS

Out of 57 cases studied, majority of the patients had serous (21 cases) or mucinous tumours (20 cases). The mean age group was 54.5 years. CD-44 expression was significantly correlated with tumour differentiation but there was no correlation found with age and histological type. In E-cadherin expression, there was no correlation with age, histological type and tumour differentiation.

CONCLUSIONS

For primary epithelial tumours, expression of CD-44 could be an indicator for tumour progression, invasiveness or distant metastasis. Poorly differentiated tumours with increased expression may be helpful in predicting disease progression. Target therapy can be employed in such cases. In case of E-cadherin which is said to be a prognostic marker, more studies help in bridging the gap between prognosis and outcome.

KEYWORDS

CD-44, E-cadherin, Immunohistochemistry, Epithelial Ovarian Tumours

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DOI: 10.18410/jebmh/2021/263

How to Cite This Article:

Valliapoyil D, Thodiyil JK, Jose L. CD-44 and E-cadherin expression in primary epithelial tumours of ovary and comparison with histological type and tumour differentiation - a cross-sectional study from Thrissur, Kerala. J Evid Based Med Healthc 2021;8(19):1380-1385. DOI: 10.18410/jebmh/2021/263

Submission 11-12-2020,

Peer Review 20-12-2020,

Acceptance 24-03-2021,

Published 10-05-2021.

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BACKGROUND

Epithelial ovarian cancer constitutes 85 % - 90 % cases of ovarian carcinoma.¹ The incidence of ovarian cancer increases with age. Over 70 % of the patients present with advanced stage of disease at the time of diagnosis.² In spite of the cyto-reduction surgery and chemotherapy, majority of the patients finally relapse. Therefore, it is necessary to explore the molecular target-directed therapies which inhibit the local invasion and distant metastasis and represent a new treatment modality.³ CD-44 is a family of non - kinase, single span trans-membrane glycoproteins expressed on embryonic stem cells.⁴ The main ligand of CD-44 is hyaluronic acid that is expressed by stromal and cancer cells. Interaction between them leads to cell proliferation, adhesion, migration and invasion. The clinicopathological impacts of CD-44 and its isoforms in promoting tumorigenesis suggest that it may be a molecular target for cancer therapy.⁵

E-cadherin, Ca²⁺ dependent cell - cell adhesion molecules⁶ play an essential role in embryonic development, morphogenesis and in the maintenance of the normal structure and function of adult tissues.⁷ It is well documented that loss of E-cadherin is essential for the development of invasive potential. The assessment of E-cadherin immunoreactivity may be a useful prognostic indicator in ovarian cancer, complementary to established prognostic factors.⁸ E-cadherin expression was a predictor of better responses to first-line platinum-based chemotherapy, platinum sensitivity and favourable clinical outcome in patients with advanced-stage ovarian cancer.⁹

Objectives

To study the immunohistochemical expression of CD 44 and E-cadherin in Primary Epithelial tumours of ovary and to compare with histological types and tumour grades.

METHODS

This is a cross-sectional study conducted in The Department of Pathology, Government Medical College, Thrissur; a tertiary care institution from January 2019 to June 2020.

Inclusion Criteria

All specimens diagnosed histologically as primary surface epithelial tumours of ovary received in the Department of Pathology, Govt. Medical College, Thrissur during the study period.

Exclusion Criteria

1. All specimens diagnosed as metastasis to ovary.
2. Samples from patients who have undergone chemotherapy and radiotherapy for ovarian neoplasms.

Sample Size Calculation

$$\text{Sample size} = Z\alpha^2 pq / d^2$$

p is sensitivity obtained from previous study by Zheng J [2017; 10 (4) :4780 - 4786]

q = {100 - p}, d = 20 % p

p = 64 %

Sample size is 54

Procedure

Four-micrometre-thick sections were made from formalin-fixed paraffin-embedded tissue and were stained with haematoxylin and eosin for diagnostic confirmation and histological grading.

For Immunostaining - Four micrometre sections were mounted on (Fisher super frost, USA) positively charged slides, then deparaffinized and rehydrated for immunohistochemical staining by CD-44 (ABCAM) and monoclonal antibodies to E-cadherin.

Heat mediated antigen retrieval is done using phosphate buffer, pH.¹⁰ Then the sections will be immersed in hydrogen peroxide (H₂O₂) to block the endogenous peroxidase activity, washed in phosphate-buffered saline (PBS) and then protein blocking reagent and incubated for 20 minutes at 37° c within humid chamber to reduce nonspecific staining. The tissue sections will be incubated with CD-44 antibodies (diluted 1 : 50) and mouse monoclonal [5H9] antihuman E-cadherin antibody (diluted 1 : 10) for one hour at 37° c. After that the slides will be kept in the refrigerator at 4° c overnight in humid chamber. The bounded antibodies will be detected by the streptavidin-biotin complex method, after an immunoreaction, the sections counterstained with haematoxylin.

Scoring System

Immunostaining for CD-44 (membrane positivity) was evaluated in a series of randomly selected five high-power fields (200 × magnification) and 100 tumour cells were counted in each field. Staining intensity score was taken as zero for no staining, one for weak staining, two for moderate staining and three for strong staining. Staining extent was calculated from the percentage of positive tumour cells. The score was given from zero to four. Zero score was given if only 0 - 5 % tumour cells are positive. Score one for 6 - 25 % positive tumour cells, score two for 26 - 50 % of cells, score three for 51 - 75 % of cells and if 76 - 100 % cells are positive score four was given.

The staining intensity score was multiplied with the staining extent score, resulting in the semi quantitative immunoreactivity score (2) that indicated the expression level of CD44.

	Final Score	Level of Expression
Semi quantitative immunoreactivity score	0 - 2	Negative
	3 - 4	Weak positive
	6 - 8	Moderate positive
	9 - 12	Strong positive

Table 1. Immunoreactivity Score for CD 44

Also, the immunostaining for E-cadherin (membrane positivity) was evaluated in a series of randomly selected five high-power fields (200 × magnification) and 100 tumour cells were counted in each field. Staining intensity score was taken as zero for no staining, one for weak staining, two for moderate staining and three for strong staining. Staining extent was calculated from the percentage of positive tumour cells. The score was given from zero to four. Zero score was given for a negative staining. Score one was given if less than 10 % tumour cells are positive, score two for 10 - 50 % of cells, score three for 51 - 80 % of cells and if more than 80 % cells are positive, score four was given.

The staining intensity score was multiplied with the staining extent score, resulting in the semi quantitative immunoreactivity score¹¹ that indicated the expression level

	Final Score	Level of Expression
	0	Negative staining
Semi quantitative immunoreactivity score	1 - 4	Weak staining
	5 - 8	Moderate staining
	9 - 12	Strong staining

Table 2. Immunoreactivity Score for E-Cadherin

The clinico-pathological characteristics of patients were gathered from the medical records and pathology reports. The diagnosis of histological type and grade were performed according to the Classification of tumours of ovary [World health organization (WHO) 2014]¹²

1. Serous ovarian tumours
2. Mucinous ovarian tumours and
3. Other epithelial ovarian tumours, which include endometrioid, clear cell, Brenner, seromucinous tumours and undifferentiated carcinoma.

Further the immunohistochemical expressions of CD-44 and E-cadherin were compared with histological types and grades of primary epithelial ovarian tumours.

Statistical Analysis

Data was coded and entered into Excel sheets and analysed using SPSS software and was described in percentages. Immunohistochemical marker expression with histological type and grade of tumour was explained in tables.

RESULTS

Age Distribution

The age of the patients ranged from 21 to 79 years and mean age was 54.5 years. The maximum number of patients were in the age group of 41 – 60 yrs. 29 cases were in less than 55 years and 28 cases above 55 years. Coming to the histological types, out of 57 cases, 21 were serous tumours, 20 cases were mucinous, 6 endometrioid, clear cell and Brenner constituted 6 and 5 respectively. Majority of the cases were well differentiated tumours - 45 cases (78.9 %).

7 cases were moderately differentiated and only 5 were poorly differentiated.

Majority of the cases show negative expression for CD-44 - 39 cases (68.4 %). 10 with weak expression and 8 cases showing moderate expression. None of the cases showed strong expression for CD-44. In case of E-cadherin expression majority of the cases, 55 out of 57 cases showed moderate E-cadherin expression (96.5 %). Two cases showed weak expression and none of the cases were negative for E-cadherin.

Types	Negative	Weak	Moderate	Total
Serous	15 (38.5 %)	6 (60.0 %)	0 (0.0 %)	21 (36.8 %)
Mucinous	16 (41.0 %)	3 (30.0 %)	1 (12.5 %)	20 (35.1 %)
Endometrioid	4 (10.3 %)	0 (0.0 %)	1 (12.5 %)	5 (8.8 %)
Clear cell	3 (7.7 %)	0 (0.0 %)	3 (37.5 %)	6 (10.5 %)
Brenner	1 (2.6 %)	1 (10.0 %)	3 (37.5 %)	5 (8.8 %)
Total	39 (100 %)	10 (100 %)	8 (100 %)	57 (100 %)

Table 3. CD-44 Expression in Different Histological Types and % within CD-44

Types	Weak	Moderate	Total
Serous	0 (0.0 %)	21 (38.2 %)	21 (36.8 %)
Mucinous	2 (100.0 %)	18 (32.7 %)	20 (35.1 %)
Endometrioid	0 (0.0 %)	5 (9.1 %)	5 (8.8 %)
Clear cell	0 (0.0 %)	6 (10.9 %)	6 (10.5 %)
Brenner	0 (0.0 %)	5 (9.1 %)	5 (8.8 %)
Total	2 (100 %)	55 (100 %)	57 (100 %)

Table 4. E Cadherin Expression in Different Histological Types and % within E Cadherin

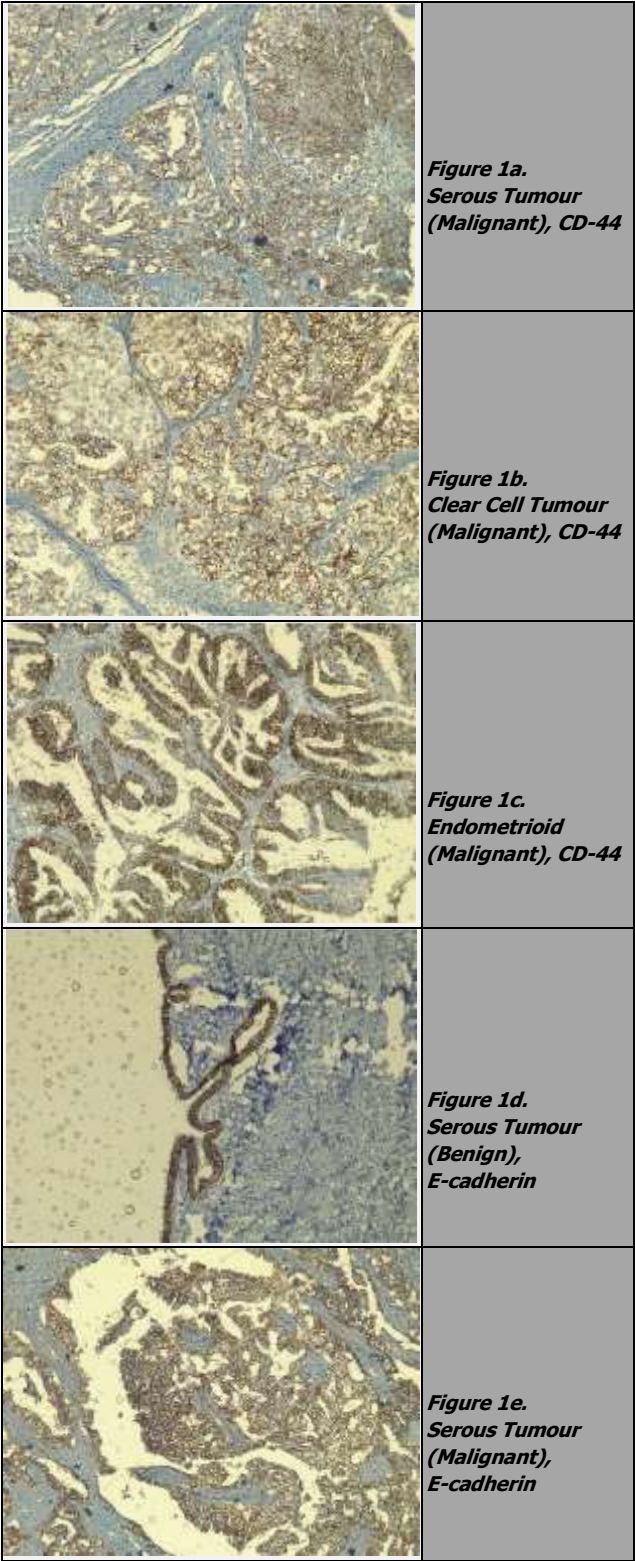
	Negative	Weak	Moderate	Strong	Total
Well differentiated	35 (89.7 %)	6 (60.0 %)	4 (50.0 %)	0 (0.0 %)	45 (78.9 %)
Moderately differentiated	2 (5.1 %)	4 (40.0 %)	1 (12.5 %)	0 (0.0 %)	7 (12.3 %)
Poorly differentiated	2 (5.1 %)	0 (0.0 %)	3 (37.5 %)	0 (0.0 %)	5 (8.8 %)
Total	39 (100 %)	10 (100 %)	8 (100 %)	0 (0.0 %)	57 (100 %)

Table 5. CD 44 Expression and Tumour Differentiation

	Negative	Weak	Moderate	Strong	Total
Well differentiated	0 (0.0 %)	2 (100.0 %)	43 (78.2 %)	0 (0.0 %)	45 (78.9 %)
Moderately differentiated	0 (0.0 %)	0 (0.0 %)	7 (12.7 %)	0 (0.0 %)	7 (12.3 %)
Poorly differentiated	0 (0.0 %)	0 (0.0 %)	5 (9.1 %)	0 (0.0 %)	5 (8.8 %)
Total	0 (0.0 %)	2 (100 %)	55 (100 %)	0 (0.0 %)	57 (100 %)

Table 6. E Cadherin Expression and Tumour Differentiation

The expression of CD-44 and E-cadherin was compared with tumour differentiation. 35 out of 45 well differentiated tumours showed a negative staining for CD-44. Six cases showed weak positivity and four cases showed moderate intensity of staining. In seven moderately differentiated tumours two were showing negative staining, four cases showed weak staining and only one case was showing moderate staining score. In case of poorly differentiated tumours, three cases out of five showed moderate intensity of staining and two cases with negative intensity score. Considering the E-cadherin expression, 43 out of 45 cases of well differentiated tumours show moderate intensity score and two showed weak intensity score. All the seven cases of moderately differentiated tumours and all five poorly differentiated tumours were showing moderate intensity of staining score.



DISCUSSION

Ovarian carcinoma is the most frequent gynaecologic malignancy resulting in cancer related death. Epithelial ovarian cancer constitutes 80 %-90 % cases of ovarian carcinoma. The lack of specific symptoms, contribute to the high mortality of ovarian carcinoma. As a consequence, more than 70 %patients are diagnosed at advanced stages with extensive local invasion and intra-peritoneal metastasis.

In spite of the cytoreduction surgery and chemotherapy, majority of the patients finally relapse and the 5-year overall survival rate was only 45 %.

Cluster differentiation 44 (CD-44), is a type of cell-surface adhesion molecule. Studies have suggested that CD44 is over expression and promotes cells migration and metastasis for human solid tumours, including breast carcinoma and ovarian carcinoma. Several studies have shown that the over expression of CD-44 enhance the capacity of proliferation and carcinogenesis in renal cancer and gastric cancer.¹³ Up to date, the relationship between CD-44 expression and clinical significance in epithelial ovarian cancer remains controversial.

Epithelial cadherin (E-cadherin) belongs to the cadherin family of calcium-dependent adhesion molecules. Loss of E-cadherin expression has been regarded as a central event in tumour metastasis, as loss of adhesion between tumour cells facilitates their ability to invade locally and to spread to distant organs. There are only a limited number of studies regarding the prognostic role of E-cadherin. Since the loss of E-cadherin has frequently been linked to increased aggressive tumour behaviour and a poor clinical outcome.

In the present study, 57 cases of primary epithelial ovarian tumours were selected. Histological types and tumour grades were determined for each of these cases based on the routine H & E sections and stained with the immunohistochemical markers for CD-44 and E-cadherin. A semiquantitative scoring system was used to grade the level of expression.

The most frequent age group is between 41 and 60 years and the age of patients ranged from 21 to 79 years. The mean age of patients is 54.5 years. This is similar to the study conducted by Zheng J, et al. which is 55.3.

Majority of the cases were serous and mucinous tumours together which contributes about 71.72 % of total cases studied. This is not consistent with the previous studies where the most common type is serous tumours.

In our study histological grades were classified as well, moderately and poorly differentiated rather than designated as Grade 1, 2 and 3. Out of the 57 cases, 45 cases were well differentiated. Number of well differentiated tumours was more in our study compared to the previous studies. Again, the selection of both benign and malignant cases in our study may have been the reason for difference from previous study.

In the present study 39 out of 57 cases (68.4 %) showed negative staining. 10 cases showed weak expression and 8 cases showed moderate expression. This is different from previous study by Zheng J, et al. where majority of the cases showed positive staining (64 %).

CD-44 and Age

In cases less than 55 years, 19 cases showed negative expression, 5 cases were showing weak staining and 5 with moderate staining. In cases above 55 years, 20 cases showed negative staining, 5 cases showed weak staining and 3 cases showed moderate staining. This data is different from previous study. The P value for our study was 0.852 and the correlation between CD-44 expression and age is not statistically significant.

CD-44 and Histological Types

15 out of 21 serous tumours, 16 out of 20 mucinous tumours, 4 out of 5 endometrioid tumours, 3 out of 6 clear cells tumours and 1 out of 5 Brenner tumours showed negative staining with CD-44. Out of 5 Brenner tumours, 3 were showing moderate staining. This finding is similar to the study by Zheng J, et al.

CD-44 and Tumour Differentiation

25 out of 45 well differentiated tumours, 2 out of 7 moderately differentiated tumours and 2 out of 5 poorly differentiated tumours showed no staining with CD-44. Here, 3 out of 5 poorly differentiated tumours showed positivity. Hence, poorly differentiated tumours show increased expression of CD-44. This is similar to previous study by Zheng J, et al.

In the present study majority of the patients (96.5 %) had moderate E-cadherin expression. Out of the 57 cases 55 cases showed moderate staining. Only 2 cases showed weak staining. This is similar to a study by Faleiro-Rodrigues C, et al. where majority (93 %) of the cases showed positivity for E-cadherin.

E-Cadherin and Age

Cases under 55 years, 28 out of 29 cases showed moderate staining and only one case showed weak staining. In cases above 55 years, 27 out of 28 cases showed moderate staining and one case with weak staining. This is similar to previous study with majority cases showing positivity irrespective of age group. This study shows no correlation with age.

E-Cadherin and Histological Type

For serous tumours, all 21 cases showed moderate E-cadherin expression. In mucinous tumours, 18 out of 20 cases showed moderate staining. All cases of endometrioid tumours, clear cell tumours and Brenner tumours show moderate E-cadherin expression. Hence, the relation between E-cadherin expression and histological type is not statistically correlated. This is similar to previous studies.

E-Cadherin and Tumour Differentiation

43 out of 45 well differentiated tumours and all moderately and poorly differentiated tumours show moderate staining with E-cadherin.

CONCLUSIONS

In the present study there was a significant relation between CD-44 and tumour differentiation, but no significant correlation was seen between the age and histological type. There was a significant difference in the expression of CD-44 between well differentiated tumours and poorly differentiated tumours. CD-44 expression is found to be increased in poorly differentiated tumours when compared

to well-differentiated tumours. In this study majority of the cases were of well differentiated type. Hence, percentage of tumours with increased CD-44 expression was less. But significant percentage of tumours among moderately and poorly differentiated tumours showed increased expression. So, this is in sync with previous study.

In this study, E- Cadherin expression was found to be positive in majority of cases. No definitive pattern of expression can be made out. No correlation could be established with age, histological type and tumour differentiation. This is similar to a study by Faleiro-Rodrigues C, et al. where E-cadherin was found to be positive in majority of cases. Many other studies have demonstrated that the loss of E-cadherin expression is frequently associated with parameters of enhanced biological aggressiveness such as poor histological differentiation, increased invasiveness, metastatic disease and a poorer survival rate in patients. But our study failed to demonstrate a significant relation between histological type and tumour differentiation. The major cause for such difference could be in the sample selection, antibodies used or techniques employed.

For primary epithelial tumours of ovary, expression of CD-44 could be an indicator for tumour progression, invasiveness or distant metastasis. Poorly differentiated tumours with increased expression may be helpful in predicting disease progression. Target therapy can be employed in such cases. In case of E-cadherin which is said to be a prognostic marker, more studies help in bridging the gap between prognosis and outcome.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

Financial or other competing interests: None.

Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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